SODIUM ION AND FATTY ACID OXIDATION IN BOVINE BRAIN MITOCHONDRIA

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In bovine brain mitochondria fatty acids are activated, in part, by high-energy intermediates of oxidative phosphorylation (Beattie and Basford, 1966). A similar activation of fatty acids by high-energy intermediates has also been proposed for liver mitochondria (Wojtczak, 1965). This paper reports an inhibition of fatty acid oxidation in brain mitochondria by the addition of Na^+ ion which has been shown by Krall (1965) to stimulate the respiratory rate of brain mitochondria in the absence of phosphate acceptor with a decrease in respiratory control. The inhibition of fatty acid oxidation by Na^+ persists in the presence of oligomycin and is reversed by increasing concentrations of fatty acid suggesting that fatty acids and Na^+ may interact with an identical high-energy intermediate of oxidative phosphorylation.

EXPERIMENTAL PROCEDURE

Bovine brain mitochondria were prepared by Method II of Stahl et al. (1963). Oxidative phosphorylation was measured polarographically in a medium containing 0.3 M mannitol, 0.01 M Tris-HCl, 0.01 M Tris-phosphate, 1 mM $MgCl_2$, 8 mg of dialyzed bovine serum albumin, 12.5 mM malate or succinate, and 3-4 mg of mitochondrial protein in a final volume of 2.0 ml. The P/O ratios were calculated as ADP/O ratios from oxygen uptake in μatoms during the active state of respiration and μmoles of ADP added. Respiratory control ratios were calculated as the rate of oxidation in the presence of

phosphate acceptor divided by the rate in the absence of acceptor.

Fatty acid oxidation was measured as previously described (Beattie and Basford, 1965). Each flask contained 0.2 mM Tris-malate, 50 μg of cytochrome c, 8 mM $MgCl_2$, 40 mM Tris, pH 7.4, 1 mM Tris-phosphate, pH 7.4, 0.04 mM octanoate 1-^{14}C containing 100,000 counts/min, 3-4 mg of mitochondrial protein and KCl or NaCl as indicated in a final volume of 2.5 ml. The flasks were incubated for 45 min at 37° in a metabolic shaker.

All reagents were prepared in glass-distilled water and where necessary, adjusted to pH 7.4 with Tris base.

RESULTS AND DISCUSSION

The rate of fatty acid oxidation in bovine brain mitochondria was inhibited 48% upon the addition of Na^+ to a system containing K^+ (Table I). When phosphate was omitted, the maximal rate of fatty acid oxidation in the presence of K^+ was lowered and Na^+ only caused an insignificant inhibition. It can also be seen in Table I that 12.5 mM Na ion in the presence of optimal K^+ (Krall et al. 1964) caused a 50% stimulation of respiration (State 4;

TABLE I

EFFECT OF K^+ AND Na^+ ON BRAIN MITOCHONDRIA

Ion	Fatty Acid Oxidation counts/min		P/O	RCR*	Respiration Rate mμatoms O/min/mg protein +ADP	-ADP
K^+	6710	1580'	2.26	2.43	8.88	3.67
Na^+	2810	1420'	1.54	1.64	9.10	5.55

Concentrations of reactants as described in the text, except 40 mM KCl and 40 mM NaCl in the studies of fatty acid oxidation. For the respiration experiments, 12 mM KCl was present and NaCl (12.5 mM) was added before ADP.

' No phosphate present * Respiratory control ratio

Chance and Williams, 1956) with a concomitant decrease in respiratory control. The addition of 50 mM NaCl doubled state 4 respiration. The rate of respiration in the presence of ADP (Chance's state 3) was essentially unaffected by the addition of Na^+; however, the P/O ratio was decreased. The rate of state 4 respiration was stimulated to the same degree by Na^+ after the mitochondria had previously reacted with ADP (state 3) and returned to the state 4 respiratory rate. The addition of oligomycin did not significantly decrease the increased rate of respiration induced by Na^+. Identical results were obtained with succinate as substrate.

An inhibition of fatty acid oxidation by increasing concentrations of Na^+ was observed in the presence or absence of K^+ (Figure 1). The increased rate of fatty acid oxidation in the presence of K^+ may be a reflection of the previously observed K^+ stimulation of brain mitochondrial respiration (Krall et al., 1964).

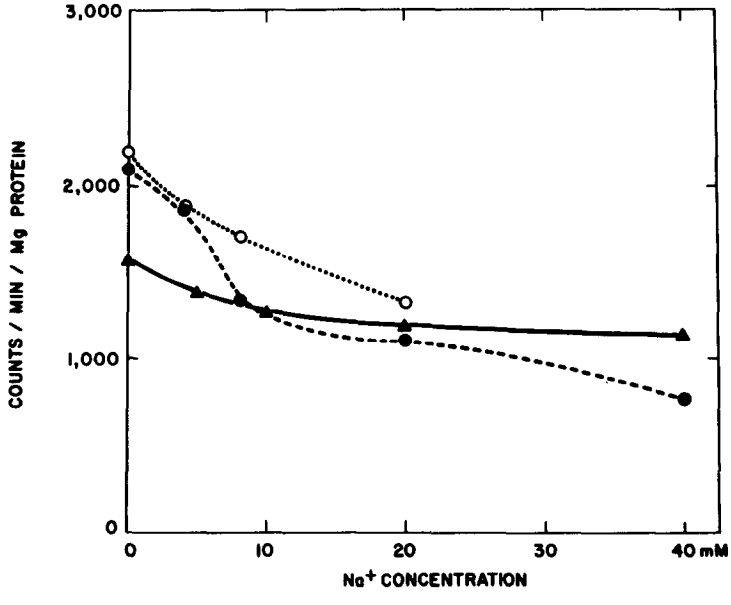

Figure 1. Inhibition of fatty acid oxidation by increasing concentrations of Na^+. ▲–▲ no ions added, ●–● 8 mM KCl present, o–o 8 mM KCl and 10 μg of oligomycin. Other conditions as described in the text.

The inhibitory effect of Na^+ on the rate of fatty acid oxidation persisted in the presence of concentrations of oligomycin sufficient to block ATP synthesis completely (Table II). The addition of ouabain, a specific inhibitor of the membrane-associated ATPase of brain which is stimulated by Na^+ (Skou, 1962; Aldridge, 1962) had no effect on the Na^+ inhibition of fatty acid oxidation by brain mitochondria (Table II).

TABLE II

EFFECT OF INHIBITORS ON Na^+ INHIBITION OF FATTY ACID OXIDATION

Ion	Control	% Inhibition	Oligomycin 4 μg/mg Protein	% Inhibition	Ouabain 1 mM
None	6660	--	--	--	--
Na^+, 20 mM	4680	30%	--	--	4610
K^+, 8 mM	7320	--	9540	--	7860
$K^+ + Na^+$	5280	28%	6730	30%	5350

The inhibition of fatty acid oxidation by Na^+ was partially reversed by increasing concentrations of octanoate (Table III). When the concentration of octanoate was increased from 6.96 to 209 mμmoles per flask, the Na^+ inhibition was decreased from 48% to 22%.

The stimulation of mitochondrial respiration in the absence of phosphate acceptor by Na^+ and the inhibition of fatty acid oxidation by Na^+ indicate that Na^+ may interact with the same high-energy intermediate implicated in the energy-linked activation of fatty acids (Beattie and Basford, 1966), since neither process is inhibited by oligomycin (Lardy, 1958). Further evidence for the identity of the high-energy intermediate involved in these two processes is the partial reversal of the Na^+ inhibition of fatty acid oxidation by increasing concentrations of octanoate. The Na^+ inhibition of fatty

acid oxidation was dependent on the presence of phosphate as well as the maximal rate of fatty acid oxidation. Krall (1965) has also observed that the Na^+ stimulation of brain mitochondrial respiration in the absence of ADP was dependent on phosphate. The lack of effect of ouabain on the Na^+ inhibition of fatty acid oxidation suggests that the effects of Na^+ on brain mitochondria are independent of those associated with the non-mitochondrial Na^+-stimulated ATPase isolated from brain.

TABLE III

REVERSAL OF THE Na^+ INHIBITION BY OCTANOATE

Octanoate Concentration μmoles/flask	mμmoles Oxidized		% Inhibition
	Control	+Na^+, 40mM	
6.96	0.85	0.419	48
16.4	1.04	0.618	40
34.8	1.32	0.940	29
69.5	1.55	1.11	28
209.0	2.27	1.77	22

The results of this report also suggest that Na^+ may be transported in brain mitochondria by an analagous mechanism to that proposed for the transport of Ca^{++} and other divalent ions in liver and heart mitochondria (Chance, 1965). The close relationship of Na^+ and K^+ concentration to nerve transmission has been well established (Skou, 1957) and it might be suggested that mitochondria are involved in these ion changes. A role of mitochondria in the transport of Na^+ ions has also been proposed for the salt gland of the herring gull (Chance et al., 1964).

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